Corticosterone response in a resident-intruder-paradigm depends on social state and coping style in adolescent male Balb-C mice

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Abstract

OBJECTIVE: Social stressors modulate the hypothalamic-pituitary-adrenal axis in rodents. However, reports on the association between corticosterone level and behavioural responses to the stressor are ambivalent. This may depend on the experimental paradigm, species- and strain-differences, duration of exposure to the stressor, but also on using either the social state (dominant or subordinate) or the coping style (proactive or passive) of an animal to correlate the corticosterone level with.

DESIGN AND SETTING: We used male Balb-C mice in a resident-intruder paradigm. Adolescent intruders (aged five to eight weeks) were transferred into the cage of an adult resident (aged about four month) for five minutes. The interactions were video-taped for behavioural analysis. Ten minutes after the encounters, intruders were sacrificed and blood samples were collected.

RESULTS: Dominant intruders showed offensive behaviours (attack, chase, tail tracking) and won most of the fights, whereas subordinate intrudes showed mainly submissive behaviours (flight, freezing) and were further classified into active and passive subordinates. Active subordinates displayed significantly more flight-behaviour than passive subordinates. Dominant intruders showed significantly higher post-stress levels of corticosterone than subordinates, which did not differ from control mice, which experienced five minutes of novel-cage exposure. Comparing all three behavioural phenotypes we found the lowest corticosterone levels in active subordinates.

CONCLUSION: Social state significantly affects the HPA-axis response to acute social stressors.

ORIGINAL ARTICLE

INTRODUCTION

The resident-intruder-paradigm, where a male intruder enters another male's territory, is a common approach to study the behavioural and physiological consequences of social stressors in an experimentally controlled setting in rodents [1]. As the resident aggressively defends its territory, a dominance-subordination relationship is established [2–4], where the social state of an individual can be deduced by its display of offensive and submissive behaviours, respectively [3,5].

Furthermore, animals can be distinguished by their coping behaviour. A proactive coping style is characterised by territorial control and high aggression levels, whereas a passive (reactive) coping style is associated with low aggression levels, immobility and freezing [1,6]. However, a proactive coping style is neither sufficient nor necessary for gaining a dominant social state, since coping strategies reflect a behavioural trait-like response-pattern of an individual, whereas social state is affected by situational characteristics and may be altered by experimental manipulations (e.g. using a highly aggressive resident). When facing a subordinate intruder, proactive residents show shorter attack-latencies than reactive residents [1,6,7]. When exposed to social defeat in a resident-intruder-paradigm (i.e. social subordination), proactive animals tend to actively flee from the opponent, whereas reactive animals adopt a submissive freezing response-pattern [1].

Reports on the association between behavioural parameters and HPA-axis activity are inconsistent. In the resident-intruder-paradigm, pair-housing, and the visible burrow system, subordinate rodents have higher corticosterone levels than dominant rodents [5,8–12]. It has even been reported, that dominants have lower post-stress corticosterone levels than controls [9]. Zhukov and co-workers [13] on the other hand find highest corticosterone levels in subdominant animals (intermediate social state), but no difference between dominant and subordinate animals of male triads. Bartolomucci and co-workers [3–4], who didn't distinguish subdominant and submissive animals in the triad paradigm, failed to find significant differences in baseline corticosterone levels between social states in a resident-intruder-paradigm on the one hand and triads of male siblings on the other hand.

After repeated social stress some subordinate animals (responsive subordinates) respond to a novel stressor with an increase in corticosterone levels, whereas others (non-responsive subordinates) do not show increased corticosterone levels [5,8,14]. The difference already occurs at the hypothalamic level, because non-responsive subordinates express less CRF-mRNA [14]. A higher increase of corticosterone- and ACTH-levels after social defeat in rats bred for low trait-anxiety compared to rats bred for high trait-anxiety has been found [15]. Blanchard and co-workers [8] suggest that non-responsiveness physiologically characterises a passive or reactive response-pattern to social stressors.

In male rodents, a proactive coping style correlates with low HPA-axis activity and reactivity, resulting in low baseline and post-stress corticosterone levels, whereas a passive coping style is accompanied by high HPA-axis activity [6,16–20]. However, some studies report no difference in corticosterone levels before and after stressful situations between coping styles [1,7]. They argue that animals with intermediate coping behaviour and aggression-levels, which can be found in some strains, have highest corticosterone levels compared to the two extreme groups (proactive and reactive animals).

The aim of this study was to directly link corticosterone levels to behavioural characteristics of intruder-animals during a resident-intruder-encounter of male Balb-C mice.

MATERIAL AND METHODS

Animals

54 adolescent male Balb-C mice (*Mus musculus*; aged 5–8 weeks) were used as intruders in a resident-intruder-paradigm. Fifteen of them were used as controls (exposed to a novel cage only) and 39 where employed for social interaction. Adult Balb-C males (aged about four months) were used as residents. Adolescents were kept in groups of four to ten in 37×44×16 cm^3 cages and housed individually at least three days before the experiments. The resident was housed individually in a 22×37×16 cage. Animals were kept in a room with large windows and, accordingly, the dark-light cycle was determined by the natural day/night cycle. Food and water was always available to them, except during social interaction. Mice were handled when cages were cleaned, but repeated handling was avoided, because of possible impairment of the stress response [21]. All mice, except controls, underwent the social stress procedure described below. 36 of the adolescents (10 controls, 26 experimental) were sacrified by cervical dislocation 15 minutes after onset of social stress and blood samples were taken through cardiac puncture for analysis of corticosterone levels as described below.

Resident-intruder paradigm

To induce social stress, a resident-intruder-paradigm was adopted [3–4,7]. Experiments were carried out in the first two hours of the dark phase of the dark-light cycle. Different residents were used, to avoid habituation to having intruders in the home cage or increasing aggressiveness throughout the tests. The adolescent intruder was introduced to the resident's home cage and the animals were allowed to interact for about four to five minutes. The interactions were videotaped. Because novel cage exposure has some effect on corticosterone levels [22], the control Balb-C were exposed to a clean empty cage of the size of the resident's home cage and allowed to explore it for four to five minutes.

Corticosterone levels

Blood samples were kept at –20 °C until used for corticosterone quantification. Corticosterone levels were assessed from full blood with OCTEIA Corticosterone HS enzyme-immunoassay (Immunodiagnostic Systems (IDS) Ltd.). Samples were diluted 1:5. Each sample was tested twice and the mean of the two measures was used for further analysis.

Analysis of agonistic behaviour

The following behavioural categories for behavioural analysis are in part adopted from Bartolomucci *et al*. [3].

- *Dominant (offensive) behaviours:* attack (motion towards the opponent leading to direct physical contact, biting), chase (going after the opponent), aggressive grooming (strong and persistent grooming of the opponent), tail tracking
- *Submissive (defensive) behaviours:* defensive upright (standing on the back-feet pushing the opponent with the forepaws), flight (escape from the opponent), freezing (immobility during physical contact with the opponent), on the back (lying motionless on the back, belly exposed to the opponent)
- *Exploration* (digging, sniffing, rearing),
- *Social investigation* (sniffing the opponents body),
- *Inactivity* (standing motionless, not in physical contact with the opponent)

For exploration and inactivity total duration (in seconds) during five minutes of interaction was assessed, for all the other behaviours frequency of occurrence was counted. Additionally the number and duration of fights as well as the number of began and won fights were recorded.

Statistical analysis

Statistical analysis was carried out using software of the SPSS Inc. (SPSS 13.0 Student version). Behavioural parameters and corticosterone levels between experimental groups were compared by t-tests, one-way-ANOVA (equal variances not assumed) and a post-hoc Scheffe-test.

RESULTS

Behavioural classification of adolescent male intruder mice

The behavioural phenotype of male intruder mice was classified into three categories (Figure 1). When intruders attacked the resident or won most of the fights during five minutes of interaction, they were considered as *dominants*. In contrast, intruders, which tried to escape from the resident, were categorized as *active subordinates*. Intruders showing neither a fight nor a flight response were regarded as *passive subordinates* (Table 1).

Table 1. Behavioural repertoire of dominant (n=14) and subordinate (n=25, 14 active, 11 passive) intruder mice during a five minute resident-intruder interaction.

BEHAVIOUR	Dominant	Subordinate	Active subordinate	Passive subordinate
Offensive behaviour				
Attack	12±6	0.2±0.4 **	0.2±0.4**	0.2±0.4**
Chase	2±2	0±0 *	0±0 **	0±0 **
Aggressive grooming	0.1±0.2	0±0	0±0	0±0
Tail tracking	17±7	1±1**	1±1**	0.4±0.8**
Submissive behaviour				
Defensive upright	13±7	11±4	13±4	10±4
Flight	2±2	13±9**	18±8**	6±5##
Freezing	0±0	2±2**	2±2*	3±1*
On the back	8±5	3±3*	4±3*	2±1**
Social investigation	1±2	1±2	1±1	2±2
Exploration (sec.)	199±42	137±50**	133±48*	142±55*
Inactivity (sec.)	1±4	20±23**	23±25*	15±20

Dominant mice demonstrated significantly more tail tracking, attack behaviour, and exploratory behaviour than subordinate mice ($p<0.001$). The frequency of flight and freezing behaviour as well as the duration of inactivity was significantly higher in subordinates ($p<0.001$). The key behavioural difference between active and passive subordinate mice is the higher rate of flight behaviour in active subordinate mice ($p<0.001$). Significance-levels shown refer to results of a t-test for comparison of dominant and subordinate animals and of a post-hoc Scheffe-test to a One-way-ANOVA for comparison of dominants, active and passive subordinates. **$p<0.001$ compared to dominants, *$p<0.05$ compared to dominants, ##$p<0.001$ compared to active subordinates

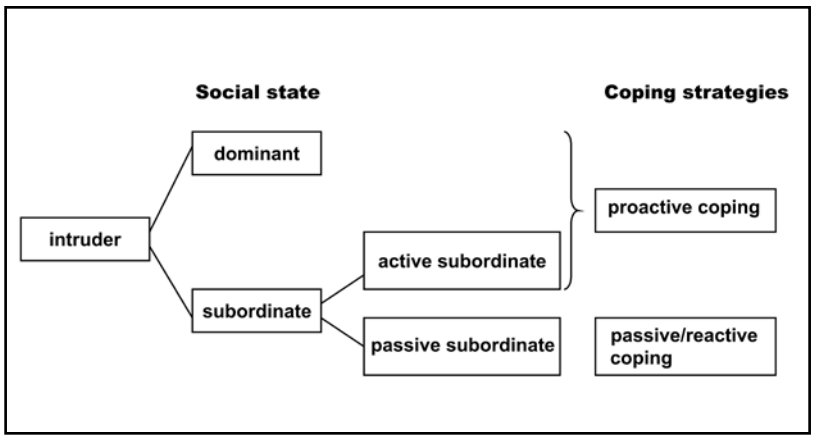

Figure 1. Behavioral classification of male intruders. Two concepts to categorise the behavioural repertoire of the intruder mouse were used: (1) the social rank order, dominant – subordinate and (2) the coping strategies, proactive and reactive. Social rank orders (dominance hierarchies) are established by aggressive encounters between a resident male and a conspecific male intruder. The dominant intruders attacked the resident and won most of the fights. Subordinate intruders did not display offensive behaviour patterns. Proactive individuals displayed the classical "fight or flight response". Consequently, dominant intruders attacked the resident whereas active subordinate intruders tried to escape from the aggressor. Passive subordinate intruders did neither offend nor physically escape from the resident.

We identified 14 dominant and 25 subordinate intruders by their display of offensive and submissive behaviours (Table 1). Dominant mice initiated 51% and won 92% of the fights they were involved in during each trial, which was significantly higher than in subordinates (t=–4.012, df=1.152, p=0.001 for initiated fights, t=–8.832, df=13, p<0.001 for winning a fight). Furthermore, exploration was higher in dominant compared to subordinate mice (t=–4.108, df=31.288, p<0.001). Subordinate mice had higher rates of inactivity (t=3.837, df=26.658, p<0.001).

Among subordinate intruders we identified 14 active and 11 passive subordinates by their display of flight/freezing-behaviour (Table 1). Freezing and inactivity didn't differ significantly between active and passive subordinates. Number (F=6.457, p=0.004) and duration (F=7.042, p=0.003) of fights were significantly lower in passive mice compared to actives and dominants. Behaviour of the resident was less aggressive (fewer attacks, less chasing) against passive subordinates.

Distinct behavioural phenotypes differ in their corticosterone level

Dominant intruder mice have significantly higher levels of corticosterone than subordinate and control animals (F=5.829, df=27, p=0.008) (Figure 2). Passive subordinates significantly differed in their corticosterone level from dominant as well as active subordinate intruders (One-way-ANOVA; F=4.372, df=27, p=0.014) (Figure 3). In a post-hoc Scheffe-test, we found a significant mean difference between active subordinates and dominants (MD=16.076, p=0.024). Figure 3 visualises that active subordinates had lowest corticosterone levels, even lower than controls, whereas passive subordinates had lower levels than dominants but hardly differed from controls. When dominant and active subordinate mice were integrated into one group (proactive coping style) they did not differ significantly from reactive mice (passive subordinates) using a One-Way-ANOVA (Figure 4).

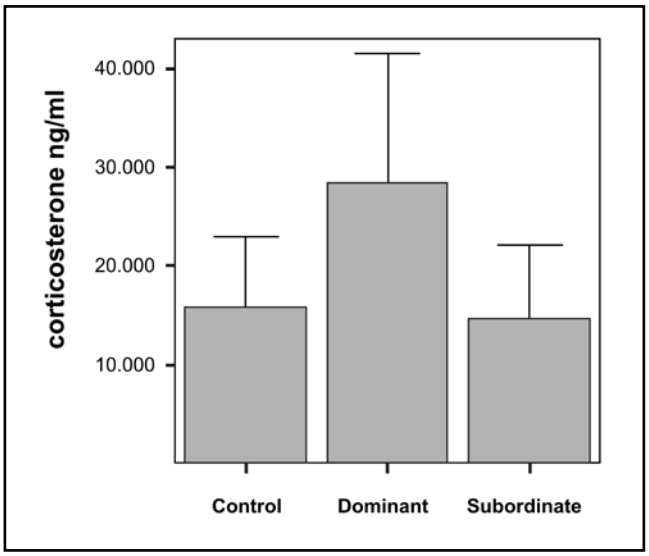

Figure 2. Mean of Corticosterone levels in dominant and subordinate intruder mice compared to controls 15 minutes after onset of a single agonistic encounter. Dominants had highest post-stress corticosterone levels, whereas subordinates hardly differed from controls.

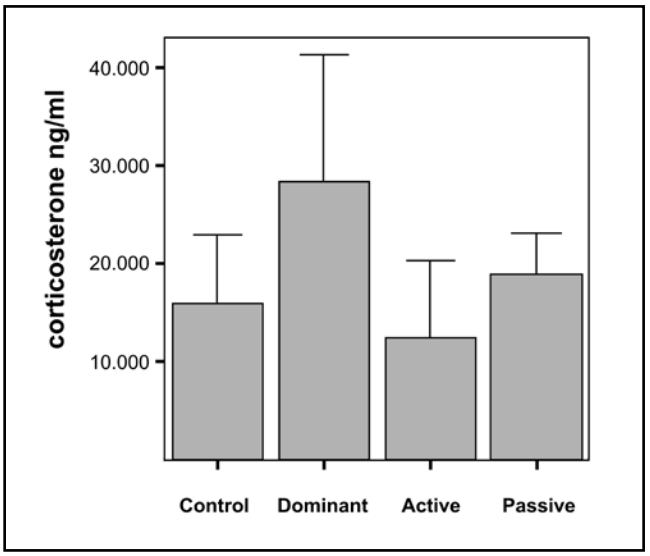

Figure 3. Mean of corticosterone levels in behaviourally differing intruder mice compared to controls. Active subordinates had lowest corticosterone levels, which were lower than in control animals. Passive subordinates had lower levels than dominants but didn't differ from controls.

DISCUSSION

We distinguished dominant, active as well as passive subordinate behavioural phenotypes in adolescent male intruders. The category "dominant or subordinate" derives from the social state of the animal, whereas active or passive subordinate phenotypes relate to individual coping style [1]. The fight-response of dominant animals can also be assessed as a proactive coping style. In our study, dominant mice showed an increased corticosterone-level compared to controls in response to social stressors, whereas subordinates did not differ from controls.

Using different paradigms, like social state or coping style, may be the difference between the detection of a correlation between corticosterone level and behaviour or not. Similar to our study, a tendency to higher corticosterone levels in dominant compared to subordinate animals has been reported for baseline corticosterone levels in adult male Swiss CD1 mice under a chronic stress paradigm [2] and in Long-Evans rats exposed to a novel stressor following repeated stress [14]. Similarly, in the visible burrow system paradigm, dominant Long-Evans rats have higher corticosterone levels than subordinate animals on day 4 [11]. Furthermore, in olive baboons it has been reported that an individual of highest aggression level had highest cortisol levels under unstable colony conditions [4]. On the other hand several studies report either a higher or an equal corticosterone level in subordinate animals compared to controls [5,8–10,12]. In the study of Hardy *et al*. [11] subordinate individuals have higher corticosterone levels than dominant individuals on day 7. On day 14, dominant and subordinate individuals have similar corticosterone levels [11].

We found the lowest corticosterone levels in active subordinate mice. Assuming that the active subordinate phenotype corresponds to a proactive coping style, whereas the passive subordinate phenotype reflects reactive coping behaviour, the difference between the subordinate phenotypes in our study is similar to findings reported on laboratory strains of rats and mice [1,6,13,18–20]. However, it is inconsistent to the assumption of a passive/reactive coping style for non-responsive subordinates by Blanchard *et al*. [8]. Grouping dominant and active subordinate intruder mice together as proactively coping animals, we did not find a difference in corticosterone levels, which is similar to those studies rejecting higher corticosterone levels in reactive animals [1,7].

Our study differs from the previous ones in two ways: we did not compare opponents, like in the cited studies, but intruder-animals, and we investigated adolescent, but not adult animals. Adolescents may differ from adults in their response to stressful situations because they have less social experience and HPA-response depends on primer social experiences during their development [23]. In male golden hamsters a two-fold-increase of post-stress cortisol levels during puberty has been reported by Wommack and co-workers [24]. They describe puberty as a period of increasing HPA-activity and also state that the development of agonistic behaviour may be influenced by glucocorticoid levels.

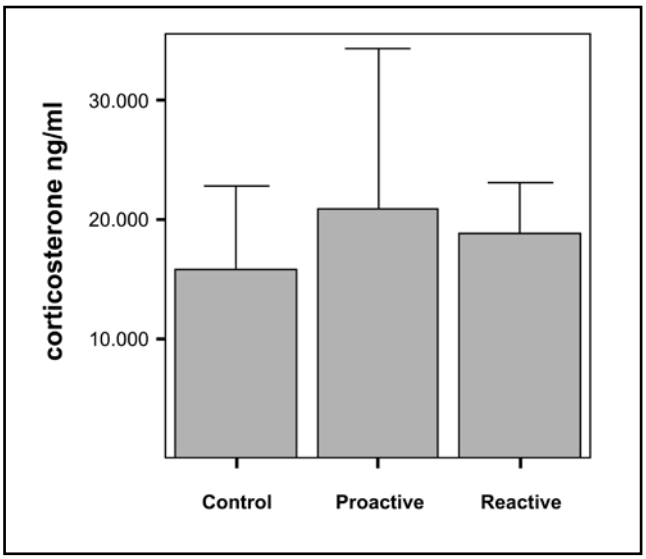

Figure 4. Mean of corticosterone levels in mice with different coping styles compared to controls. The three groups do not differ in their activation of the HPA-axis as measured by corticosterone levels.

It therefore seems that varying behavioural definitions of social state and coping style in different experimental paradigms may be the source of inconsistent results. The actual increase in corticosterone levels during an interaction is influenced by situational characteristics, such as social state or colony-stability [6], as well as individual personality traits, such as coping behaviour.

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